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ON THE NATURE OF THE STIMULUS CAUSING THE CHANGE OF FORM AND STRUCTURE IN *PROSERPINACA PALUSTRIS.*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XXXVII.

By WILLIAM BURNET McCALLUM.

(WITH TEN FIGURES)

ALTHOUGH heterophylly in amphibious plants has long been a subject of observation and experiment, as yet we are in ignorance of the real factors that determine which type of leaf such a plant shall assume. The environment of a submerged water plant is certainly a complex one, and there has not been anything like a definite analysis of it. If we are to understand the behavior of a plant when submerged, it is clear that there must be a definite knowledge of the various factors concerned, and of the influence of each one upon the plant. The literature on the subject in the main discusses such topics as water medium, diminished light relations, different oxygen and carbon dioxide conditions, buoyancy, nutrition, and the like; but such terms are general and indefinite, and convey little real information as to the actual factors acting upon the primordial cells of the plant. The demands of physiology can only be satisfied by a detailed analysis of all the possible factors, each of which is a complex in itself, and a determination of the direct action of each resolved component upon the protoplasm of those portions of the plant involved. Of course such an aim is only ideal and at present cannot be realized, but it is, nevertheless, the ultimate goal to be reached before we can read the plant in terms of its environment.

In the hope of obtaining some information upon this question, experiments were begun on *Proserpinaca palustris* in the autumn of 1900, and are still in progress. The plants used have been grown from seed or obtained from the low-lying lands about

Chicago, where they grow abundantly. The work has all been done at the Hull Botanical Laboratory of the University of Chicago, most of the experiments having been carried on in the conservatory. The results are given here only in outline. A more detailed account will appear later, when other phases of the work now in progress will be presented.

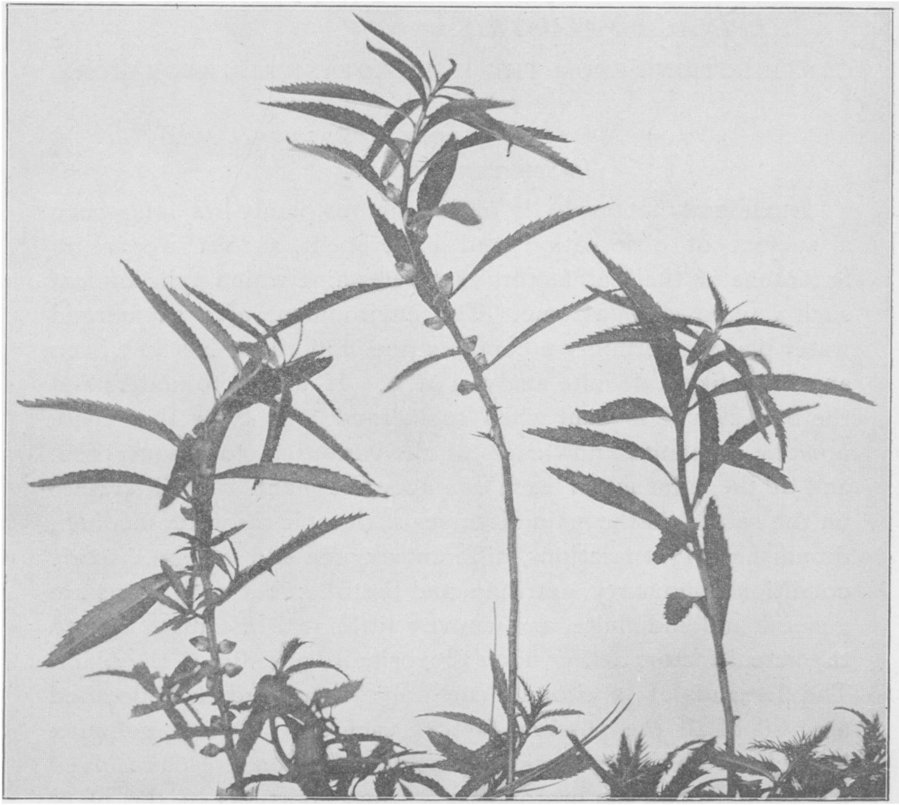

FIG. 1.—Typical air plants, bearing seed.

Proserpinaca palustris grows in low, swampy situations usually flooded in the spring and early summer, and subject to occasional submergence throughout the season. The shoots develop equally well in air and water and present two very distinct forms. When grown in the air the leaves are lanceolate in outline, 5 to 5.5^{cm} long and 7 to 8^{mm} broad, with margins rather finely serrated (*figs. 1* and *3*). The structure is that common to air leaves—that is, thick cuticle, abundance of stomata on the under surface, strong development of palisades, and a well-developed vascular system (*fig. 5*). In the stem there are the usual vascular and mechanical tissues, cortex, etc. The whole form and structure is that of a typical aerial shoot.

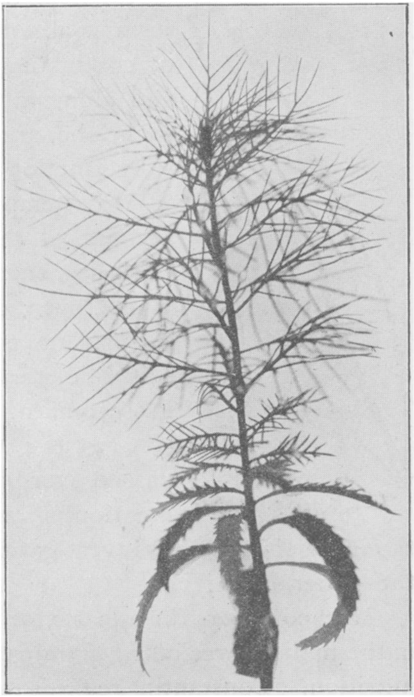

FIG. 2.—Water form in nutrient solution.

When submerged the shoots that develop are entirely different. The leaf is finely dissected, consisting of a slender central rib, with from three to five thread-like lobes on each side (*figs. 2*, *4*, and *8*), which are often only about 0.2^{mm} in diameter, and may become 2^{cm} long. These filamentous divisions are cylindrical in cross-section, and show almost an entire absence of stomata; the epidermal cells are very small and without any trace of cuticle, the outer walls being thin and delicate (*fig. 6*). There is an absence of any sort of differentiation in the mesophyll, no trace of palisades appearing. The chlorophyll has a marked peripheral placement, the outer cells, which are spherical, being packed full, while the inner ones have almost none. The mesophyll is very loosely arranged, and with a copious development of air chambers throughout. The dry weight is just one-tenth that of the air leaf. There is an entire absence of any sort of mechanical tissue or of xylem, both of which are conspicuous in the air leaves. The vascular system is represented only by a weak development of phloem. The stem of the water form is traversed by large and conspicuous air chambers that connect with the leaves and the roots. The mechanical tissue so conspicuous in the air form is absent altogether, nor can any trace of conducting tissue be seen. In the axils, and along the leaf margins, and terminating each lobe, are gland-like structures that possibly function as hydathodes.

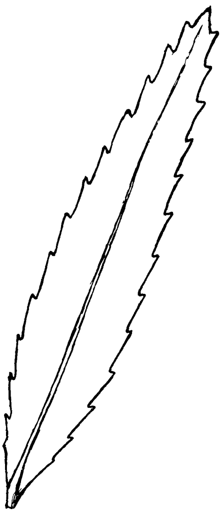

FIG. 3.— Aerial leaf.

No attempt will be made here to give any account of the literature. A good deal of work has been done upon other amphibious plants, but the object of this paper is merely to present some results of experiment on Proserpinaca, and the literature will be passed over by merely mentioning a few of the more conspicuous investigations upon the general subject.

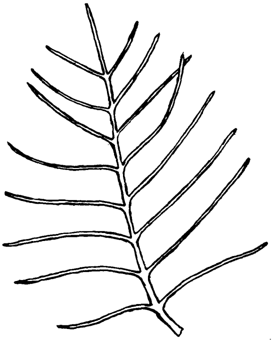

FIG. 4.— Submerged leaf.

Mer[1] considers the aquatic form as due in the main to weakened illumination and nutrition. Constantin[2] regards it a result of the weakening of vegetative activity. Schenck[3] considers it largely a question of the relation to light and to food absorption. Goebel, while recognizing the complexity[4] of the conditions, regards light, or rather the lack of light, as the chief factor. Wachter[5] substantially agrees with Goebel.

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[1] MER E., Des causes qui modifient la structure de certain plantes aquatiques végétant dans l'eau. Bull. Soc. Bot. France **27**: 194. 1880.

[2] CONSTANTIN, J., Observations critiques sur l'epiderme des feuilles des végétaux aquatiques. Bull. Soc. Bot. France **32**: 83. 1885.

Recherches sur la structure de la tige des plantes aquatiques. Ann. Sci. Nat. Bot. VI. **19**: 287–331. *pls. 14–17.* 1884.

Etudes sur les feuilles des plantes aquatiques. Ann. Sci. Nat. Bot. VII. **3**: 94–162. 1886.

[3] SCHENCK, Vergleichende Anatomie der submersen Pflanzen. 1886.

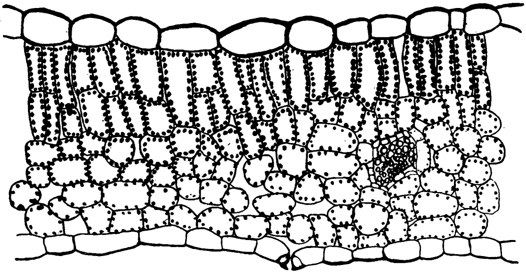

FIG. 5.— Cross section of aerial leaf.

There seems to be thus an almost universal tendency among writers to attribute the difference in structure between aerial and water forms to differences in light or nutrition, especially the former. These terms, however, are very general, especially nutrition, which involves so much as to mean little in the way of explanation. Ultimately, there must be a critical analysis of the conditions present in the water medium, of the individual factors there that can act upon the growing cells, and the effect of each of these on the behavior of the primordia.

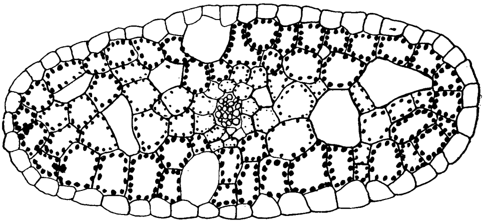

FIG. 6.—Cross section of submerged leaf.

Every living cell in a plant is surrounded at all times by a thin membrane of water through which all substances entering the cell must pass in solution. No substance, be it salt, organic compound, or gas, can enter or leave the cell in any other way than by diffusion as a solute through this film of water. When a plant is submerged this membrane is extended, its thickness being the depth beneath the surface of the water; and all gases, etc., and light which reach the cell from the exterior must pass through this increased thickness of water. What different conditions are here introduced, capable of modifying the life-phenomena of the cell? Until we have a definite knowledge of what these conditions are, *i. e.*, of the different physical conditions involving the entrance of gases, salts, or light through water measured by fractions of a millimeter or by centimeters, we cannot hope to know much about the difference in the environment of the cell in air and in water.

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[4] GOEBEL, K., Pflanzenbiologische Schildenungen. Part II. 1891.

Ueber die Einwicklung des Lichtes auf die Gestaltung der Kakteen und anderer Pflanzen. Flora **80**: 96–116. *figs. 5.* 1895.

Ueber der Einfluss des Lichtes auf die Lestaltung der Kakteen und anderer Pflanzen. Flora **82**: 1–13. *figs. 4.* 1899.

[5] WACHTER, W., Beitrage zur Kenntniss einige Wasserpflanzen. Flora **83**: 343–348. 1897.

Merely for the sake of convenience in discussion, we may arrange what seem to be the possible factors acting upon aquatic plants under the following heads: nutrition, light, temperature, gaseous content of water, substances in the water, and what seems to have altogether escaped notice, the influence of the water itself, either acting as a contact stimulus (stereotropism), or by diluting the cell contents and protoplasm. Of course, such a classification of factors is essentially superficial. It would be quite erroneous, for example, to consider nutrition apart from light which involves photosynthesis, or from temperature, or gas exchange, or absorption of salts; but it will enable us to discuss the matter more systematically and with greater clearness. It is to be understood that the experiments are given here only in outline.

1. LIGHT.—A large number of experiments were conducted. Plants were grown in the strongest sunlight available, and in darkness, and in all stages between, the experiments always being duplicated in water and in air. Plants were also grown in various depths of water. The results were always uniform; the light apparently having no influence whatsoever on the character of the developing shoot. When in the water it produced the water form, and in the air the air form, regardless of the illumination. Plants with their tips the merest fraction of an inch beneath the surface, in both strong and weak light, produced the water form; and those with their tips just above the water produced the air form, no matter what the illumination (*fig. 7*).

These experiments were done many times, with many plants and always with the same result. Objection may be raised that light passing through water is not quite the same as that obtained by shading; and to make sure, plants were grown under double walled bell-jars with the space between the walls filled with water, so that all light coming to the plants had to pass through 2 to 3^{cm} of water. The behavior here was precisely the same as in the other cases, *i. e.*, if the growing tip was in the air, the air form was produced, even when the experiment was conducted in very subdued light. When conducted in bright sunlight the only difference observed was a more vigorous growth. These experiments show that the illumination neither directly, nor indirectly by any subsequent changes that it may involve, can play an essential part in the stimulus to the water form.

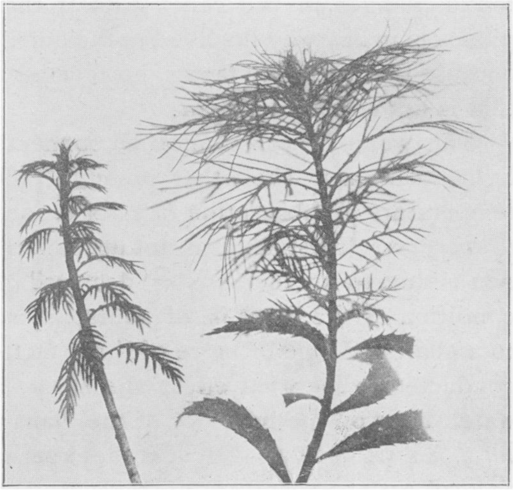

FIG. 7.—Submerged plants in very good and very poor illumination.

2. NUTRITION.—One must hesitate before using this term. It is so very vague and indefinite that its chief advantage lies in its convenience in covering a mass of ignorance. It involves the absorption and the use of oxygen, the absorption of carbon dioxide, and the formation of carbohydrates, and hence the action of illumination; the formation of proteids and hence the absorption of the various salts, also the migration of food and other substances, and all the factors that control this, the toxic and other effects of various ions which may be present. In

fact, the entire list of chemical and physical phenomena occurring within the cell, diverse and complicated as these are, and all the factors which can influence them are involved in nutrition. Disturbances in these nutritive relations may be invoked by the slightest increase or decrease in any of the factors concerned. The change in concentration of a single ion may result in profound changes in the cell. And if the water form of Proserpinaca is in any way involved in the nutritive relations it must be because there is some factor, however slight, acting in the water that is not acting in the air.

This we have seen cannot be connected in any way with the light relations. The other possible factors are the relation to temperature, to absorption of salts or ions, to carbon dioxide, or to oxygen. To state, as is not uncommon, that such a phenomenon is due to an increase or decrease of the general nutritive condition of the plant is, of course, merely to say that it is due to a change in one or more of these factors. Experiments were conducted to see what effect these, as they are present in the water, have on the behavior of the plant when submerged.

3. TEMPERATURE.—Most of the experiments were conducted at the ordinary temperature of the conservatory. During the winter some were kept at temperatures so low as almost to inhibit growth, this occurring very slowly. Plants also were kept near the hot water pipes at a temperature so high as occasionally to kill some. In the summer the highest temperatures in the sunlight were used. In all of these cases duplicate experiments were conducted, some in air and some in water, so that the temperature for the two was the same. Some were entirely submerged, some with their tips just above water, some entirely in the air, and some in the air with their tips bent over into the water. In all cases the form of the leaf assumed had no relation to the temperature; as long as any growth occurred the growing points beneath the water produced the water form. In one case two tips at precisely the same temperature were not a centimeter apart, one beneath water and the other above with all the rest of the plant submerged, and the former produced water leayes,

while the latter developed the air form. In general, the *optimum* temperature for the two forms, so far as could be observed, is about the same. This indicates that temperature, in itself, is not a factor in determining which form shall be produced.

4. SALTS.—The relations of carbon dioxide and oxygen to a possible stimulus will be discussed later under another head, as well as the question of food salts. Plants were grown in distilled water, in tap water, and in nutrient solutions. Some were entirely submerged, with their roots in the soil; others had the soil washed away from the roots, and shoots and roots submerged in the water. Some in both distilled and tap water were submerged except the very tips, which were in the air. The same was done in various strengths of nutrient solution. All of these were repeated in strong and weak illumination. The results were invariably the same. The finely divided leaf, with the aquatic type of structure in the leaf and shoot, always occurred when the growing tips were submerged, it mattered not whether in distilled water or in nutrient solutions, or in strong or weak illumi-

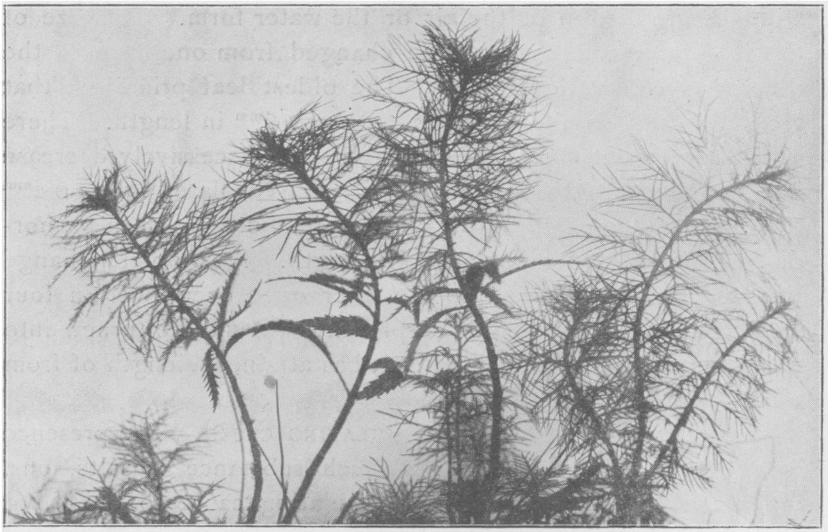

FIG. 8.— Plants after being submerged two months.

nation; while when the tips were in the air the broad leaves were produced whether the rest of the plant was in nutrient solution or distilled water. *Fig. 2* shows a plant grown in nutrient solution, and *fig. 8* shows similar plants in water.

This seems to throw out the general conditions of nutrition, so far as the salts are concerned, as having anything at all to do with the production of the air or the water form. The size of the leaf primordia that can be changed from one form to the other may be mentioned here. The oldest leaf primordia that are still folded in the bud average about 6^{mm} in length. There are usually about nine primordia, and they successively decrease in size, the smallest one that can be readily isolated being 0.2^{mm} long. On the creeping shoots there are about eighteen primordia, the smallest being 0.06^{mm} in length. A complete change from the one form of leaf to the other often occurs within four internodes, which means that a primordium may be turned into either an air or a water leaf after it has attained a length of from 3 to 4^{mm}.

5. SALTS, IONS, ETC., AS A STIMULATING CAUSE.—The presence in the water in solution of any such substance as salts, ions, organic acids, or the like, as a stimulating cause to the water form is conceivable. Its possibility, however, is precluded by the fact that the behavior is the same in redistilled water, and to a certain extent in a saturated atmosphere.

6. RELATIONS TO CO_2 AND TO O_2.—The gaseous content of the water differs from that of the air. The air contains, per $1{,}000^{cc}$, 209^{cc} O and 0.04^{cc} CO_2; while water at 20° C. contains, per $1{,}000^{cc}$, 5.7^{cc} O and 0.3^{cc} CO_2, about $\frac{1}{37}$ the amount of O, and 75 times the amount of CO_2. But the rate of diffusion in the water is much slower than in the air, and the cell may be able to use both gases much faster than the slower diffusion will supply them. The leaves cannot be lacking in oxygen during daylight, for the evolution of bubbles of this gas indicates the excess of the amount generated over that used. But the gas will tend to pass off from all surfaces of the cell equally, and as much per unit of surface will pass into the large and numerous air cham-

bers as is given off from the external surface, and the combined surfaces of the former are often greater than the latter. As these air cavities are continuous through the leaf and stem up into the growing parts, it follows from the laws of diffusion that these latter when illuminated must be supplied with an atmosphere rich in oxygen. This internal atmosphere will vary under different conditions, for during the night, or at times when photosynthesis is not active, the excess of O_2 will diffuse out and CO_2 will accumulate. The gas obtained from stems of *Proserpinaca palustris* growing in deep water and after several days of dull weather contained O_2 4.4 per cent., N 35.6 per cent., CO_2 10 per cent.; and the gas removed from the stems of Nuphar during bright sunshine consisted of O_2 35 per cent., N 65 per cent., CO_2 0.6 per cent.

In the case of CO_2, the slowness of diffusion may counteract the higher percentage in the water. In a perfectly saturated atmosphere, however, where the supply of CO_2 and O is normal, the plant for a long time continues the development of what is essentially the water form. When grown in sugar (lactose) solution the water form is still produced, though in these experiments it has not yet been determined just how much growth has occurred before fermentation has destroyed all the sugar. It is quite possible too that the plant is unable to use the sugar as food.

Plants were grown under water in glass vessels containing atmospheres of different O_2 and CO_2 pressures as follows: (1) one volume of water to an atmosphere containing an equal volume each of CO_2 and O_2; (2) one volume water to an atmosphere of three equal volumes of CO_2 and one volume of air; (3) one part by volume of water to three parts by volume of O_2 and one third part CO_2. For the last experiment the CO_2 pressure was about five hundred times and the O_2 approximately five times as great as that of ordinary atmosphere; while in the second case the O_2 pressure was diminished one-third and the CO_2 pressure increased 250,000 times. In all cases the pressure of the mixture was approximately 760^{mm} mercury; and the experiments were continued long enough to insure the saturation of the water at these pressures, which in the case of O_2 takes about a couple of weeks.

The results of the experiments were that in every case the water form was produced. This shows that the lack of CO_2, at least, cannot be involved in the stimulus to the formation of the water form, for the amount that diffused into the plant was, at least in the one case, certainly greater than that which it gets from the normal atmosphere. Of course it is possible that the large amount might induce abnormal or pathological changes which would prevent the normal development, but the two smaller quantities produced the same results. While an increased pressure of five times the normal O_2 pressure still results in the water form, it is possible that still higher pressure might act differently. This is not probable, however, for two reasons: (1) the plants

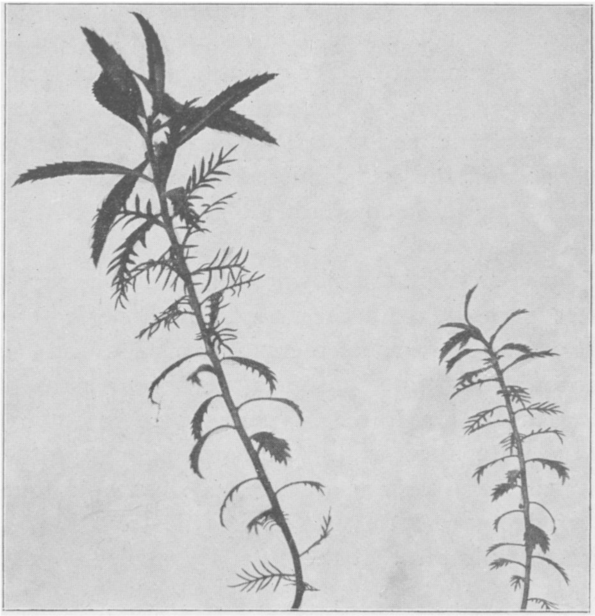

FIG. 9 — Plants grown first in water, then in air, then in water, then in air.

during these and other experiments during the day gave off O_2 freely, and this could only occur when the cells are making more than they can use and the protoplasm is supersaturated, probably even more than in the air where the O_2 passes off with less resistance; (2) because of the behavior in moist air where the plants have the same amount of oxygen as usual in the atmosphere.

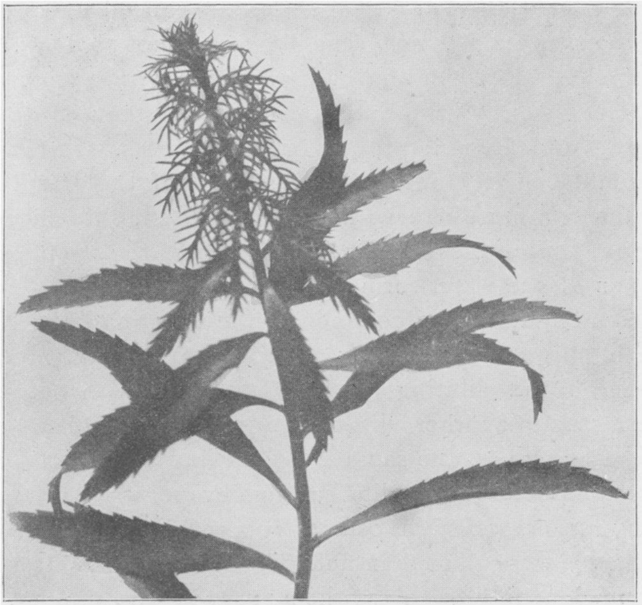

FIG. 10.— A plant whose upper leaves have developed in saturated air.

7. MOIST AIR.—The water medium necessarily has the effect of entirely checking transpiration, so that its passage from the cells is entirely prevented, and the cells and protoplasm are filled to their utmost capacity with water. This condition, to a certain extent, can be imitated by growing the plants in an atmosphere saturated with water vapor. Such a thing as a perfectly saturated atmosphere, so far as the plant is concerned, is necessarily impossible, for the temperature of the plant is always slightly higher than that of the surrounding medium, and so evaporation

from it must occur. In a large number of experiments considerable variation in this respect was noticed. Some plants when transferred from the air into a moist chamber showed little change, while others developed leaves somewhat intermediate between those and the water leaves. Some, where extra precaution to keep the atmosphere as near saturation as possible and free from changes of temperature, produced essentially the water form, as in *fig. 10*. The lobes are not so long, but the form is the same. In the case of plants grown from seed in moist chambers, several have reached as many as thirty-five internodes and are still producing water leaves. In these cases the amount of CO_2 and O_2 is the same as if the plants grew in the ordinary atmosphere, so that here, at least, differences in the amount of these gases cannot be any part of the stimulus to the formation of the water form.

The essential feature common to the water and the moist air is the inhibition of transpiration and the consequent choking of the cells and diluting of the protoplasm with water. This can be tested in another way, *i. e.*, by growing the plants entirely under water and at the same time drawing the water out from the protoplasm; or in reality causing evaporation, or transpiration, by means of high osmotic pressure. Plants producing water leaves were placed in nutrient solutions of a strength not quite sufficient to plasmolyze them; also in very dilute solutions made up to the same osmotic pressure with KCl and also $Ca(NO_3)_2$. These were allowed to evaporate down, becoming gradually stronger. When they reached a strength of solution equal to about a ½ N salt solution the water leaves ceased to form and the air type of leaf appeared. This shows that even under water, if sufficient water be drawn from the protoplasm, the air leaf will be formed. As the amount of light, CO_2, and O_2 is the same here as in the water, and the water type is not produced, it is evident that these form no part of the stimulus to the formation of the water form. Further experiments along this line are still in progress, with other salts and also with non-electrolytes.

8. CONTACT STIMULUS.—The young primordia in the submerged plants are intimately surrounded by water, and it is possible that there might be some contact influence here which checks lateral growth and causes the elongation of the lobes. Contact stimulus (stereotropism) is well known among the lower animals, as, for example, where the rhizoids of some of the hydroid polyps develop as a response to a stimulus of contact from a foreign body. That this cannot be the cause, or any essential part of the cause, is shown by the fact of the formation of the water form in moist air, and also from the facts just mentioned, where this contact is present but the air form is produced.

As to the purely anatomical features of the plant, experimental work is being carried on. A large mass of facts regarding the appearance of the various anatomical features under the diverse conditions of experiment has accumulated and will be presented later. One point only need be mentioned. Besides the positive anatomical characters of the water form, there are what we may perhaps term negative characters, *i. e.*, an absence of certain characters that usually accompany the air conditions. These are the absence of such structures as lignified and cuticularized tissues, stomata, mechanical tissue, and conducting tissue. Experiments are under way to determine to what extent such tissues are dependent upon definite external stimulating causes, and to what extent they will respond if these be supplied under the water.

The results of the experiments so far seem to justify the assertion that the stimulus to the development of the water form in the plant under discussion is not involved in the light relations, in the nutritive conditions, temperature, the gaseous content of the water, nor contact stimulus. The only factor which is constant in all cases where the water form develops is the checking of transpiration and the consequent increased amount of water in the protoplasm. When the protoplasm of the primordial cells is in that condition of dilution which accompanies the absorption of a large amount of water, the nature of the growth and the orientation of the cell division is such as to produce the water form, while those physical and chemical conditions resulting from a partial withdrawal of water by evaporation (*i. e.*, an increased density of the protoplasm) result in that sort of cell behavior which produces the air form of the plant. Just why this difference in the density of the protoplasm should result in such a different behavior of the cells is a question which this paper does not attempt to discuss. The answer can only come with an increased knowledge of the mechanics of cell division, about which at present we know almost nothing.

One other phenomenon must be mentioned. The results given were all obtained from plants collected outside, which were usually producing the air forms, or else from seedlings grown inside. When some of these had been kept submerged it was noticed that after several months they started to produce the air type of leaf. This only occurred in some cases, and even those in the same aquarium behaved differently. Also in the case of those outside, which were submerged all winter, during May and June many of them succeeded in producing air leaves, though none were able to develop flowers. It would seem here as if some of the plants after a time became accustomed to the stimulus and refused to respond. Or it may be that as only the air form is capable of fruiting, in the effort to produce flowers the plant has the ability of self-adjustment to its conditions and develops the air form in spite of its environment. Examination of these primordia showed that the cells are smaller and the protoplasm denser and less distended with water. It is possible that the protoplasm is able to adjust itself, perhaps by the expulsion of water, into that condition in which it exists when in the air. This, of course, is speculation, but investigations are under way, which it is hoped will give us some information on the subject.

In conclusion, I have pleasure in acknowledging my indebtedness to Professor John M. Coulter, Professor Charles R. Barnes, and to Dr. B. E. Livingston, for much kind assistance and advice.

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